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PRINCIPAL INVESTIGATOR: Carla Y. Pumphrey, Ph.D.
Ralph D. Sanderson, Ph.D.

CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences
Little Rock, Arkansas 72205

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Carla Y. Pumphrey, Ph.D.
Ralph D. Sanderson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Arkansas for Medical Sciences
Little Rock, Arkansas 72205

E-Mail: pumphreycarlay@uams.edu

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The goal of the proposed work is to evaluate the cell growth inhibitory affects of heparan sulfate proteoglycan (HSPG) gene therapy. The HSPG syndecan-1 has proven to be a powerful inhibitor of tumor cell growth and loss of expression has been correlated with poor prognosis in some cancers. Therefore, two c-myc tagged human syndecan-1 gene cassettes have been constructed for expression of syndecan-1 on the cell surface or in a soluble form. These constructs have been transfected in MDA-MB-231 cells and cloned cell lines have been established. The MDA-MB-231 cells that express increased cell surface syndecan-1 or soluble syndecan-1 grow at the same rate as parental cells in MTT cell viability assays. However, expression of increased syndecan-1 or soluble syndecan-1 improves anchorage-independent growth. The results of invasion assays through matrigel-coated transwells demonstrate that cells expressing either syndecan-1 transgene have improved invasive capacity as compared to controls. Thus, surprisingly, increased expression of syndecan-1 in breast cancer cells may contribute to enhanced growth and metastasis.

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INTRODUCTION

Heparan sulfate proteoglycans (HSPGs) regulate normal and cancer cell behaviors by binding growth factors, and by mediating cell adhesion and invasion. Current data strongly support the idea that HSPGs are a new class of tumor suppressors. Studies demonstrate the anti-tumor growth properties of three HSPGs, specifically syndecan-1, glypican-1, and betaglycan. Treatment with purified syndecan-1 produces strong growth suppression in multiple myeloma cell lines, a poorly differentiated squamous cell carcinoma cell line, human and murine mammary tumor cell lines, but not normal cell lines. In addition, treatment of multiple myeloma cells with purified syndecan-1 induces apoptosis. Many tumors display an alteration in cell surface HSPG expression. When syndecan-1 is lost from the surface of mammary epithelia, the cells lose epithelial morphology, invade collagen gels and show characteristics of neoplastic growth. When transfected with the syndecan-1 gene, transformed mammary epithelial cells regain morphology and lose neoplastic growth characteristics. *In vivo* experiments demonstrate reduced tumorigenicity of syndecan-1 or glypican-1 expressing multiple myeloma cells and betaglycan expressing breast cancer cells. We propose that HSPGs are excellent candidates for gene therapy applications for the treatment and possible eradication of breast cancer. Therefore, the purpose of the proposed work is to evaluate the affect of HSPG expression on breast cancer growth and progression. This work represents a novel use of HSPGs genes as anti-cancer therapy.

BODY

Task 1. Develop breast cancer gene therapy and evaluate *in vitro*.

- Engineer epitope tag containing human HSPG gene constructs for cell surface expression or secretion.
- Transfer epitope tagged HSPG gene construct into breast cancer cell lines and establish cloned cell lines.
- Determine the affects of HSPG production on breast cancer growth.

As was shown in the year one progress report, syndecan-1 is expressed on the cell surface of breast cancer cell lines including MCF-7, MDA-MB-231, MDA-MB-435, MDA-MB-436 and Hs578t at similar levels. Therefore, to distinguish native syndecan-1 from syndecan-1 expressed from a transgene, a c-myc tag was incorporated into the human syndecan-1 gene construct as describe in the second year progress report and the appended manuscript in preparation. Full-length and truncated c-myc tag containing syndecan-1 gene constructs have been produced and cell lines stably expressing these transgenes have been established. Expression of the full-length syndecan-1 gene cassette produces cells with increased levels of cell surface syndecan-1. The truncated gene cassette is devoid of the transmemebrane and cytoplasmic tail DNA sequence therefore expression produces a soluble syndecan-1 proteoglycan that is secreted from the cells. Cell lines expressing these transgenes have been evaluated for growth *in vitro*, growth in

soft-agar and for invasive capacity. The results are described in detail in the appended manuscript entitled "Increased syndecan-1 expression promotes anchorage-independent growth and invasion of MDA-MB-231 breast cancer cells".

Task 2. *In vivo* analysis of breast cancer gene therapy.

- Evaluation of tumor burden produced by syndecan-1 transgene expressing breast cancer cells in nude mice.

Experiments to evaluate the growth properties or the metastatic potential of the MDA-MB-231 cells transfected with vector only (control cell line) or expressing either of the above described human syndecan-1 constructs have been performed in nude mice. Each cell line, suspended in matrigel, was injected into the mammary fat pad of nude mice. After 2 months, only very small tumors are observed regardless of syndecan-1 expression profile. Results of intra-cardiac injection of each cell line indicate no difference in metastasis to bone.

KEY RESEARCH ACCOPLISHMENTS

Task 1.

- Full-length and truncated syndecan-1 gene constructs containing a c-myc tag have been produced by PCR amplification and cloned in plasmid vectors.
- The two c-myc tagged syndecan-1 constructs or an empty vector have been transfected into MDA-MB-231 and MCF-7 cell lines.
- Clones of each MDA-MB-231 cell line including vector only, full-length and soluble syndecan-1 expressing cells have been produced by limiting dilution.
- Increased expression of cell surface syndecan-1 has been confirmed by FACS analysis.
- Secretion of soluble syndecan-1 has been confirmed by dot blot and western blot analysis of conditioned media.
- Growth characteristics of cloned cells compared to parental have been evaluated by MTT assay.
- Anchorage-independent growth of each cloned cell line has been evaluated.
- Invasive capacity of the cell lines expressing increased syndecan-1 on the cell surface or in a soluble form has been determined.
- A manuscript reporting the results of the above experiments has been prepared.

Task 2.

- The tumorigenicity of the cloned MDA-MB-231 cell lines transfected with vector only or expressing increased amounts of full-length syndecan-1 or soluble syndecan-1 has been determined.
- Metastatic potential of MDA-MB-231 cells expressing increased amounts of cell surface syndcan-1 or soluble syndecan-1 has been determined.

REPORTABLE OUTCOMES

- A manuscript entitled: "Increased syndecan-1 expression promotes anchorage-independent growth and invasion of MDA-MB-231 breast cancer cells", is in preparation.
- An abstracted entitled: "Overexpression of cell surface or soluble syndecan-1 promotes growth and invasion of MDA-MB-231 breast cancer cells", has been submitted for presentation at the ERA OF HOPE Department of Defense breast cancer research program meeting.

CONCLUSIONS

The objective of the proposal is to transfer HSPG gene constructs into breast cancer cells lines and to test the ability of these tumor suppressor genes to slow growth and possibly eradicate tumors. To determine if increased expression of syndecan-1 on the cell surface or the production of soluble syndecan-1 affects breast tumor cell behavior, the MDA-MB-231 cell line was transfected with vector or either of two syndecan-1 gene cassettes and cloned cell lines were established. One cassette encodes the full-length cell surface form of syndecan-1 and other encodes a truncated molecule composed of the ectodomain which is secreted from cells in a soluble form. MTT analysis shows little difference in cell viability at several time points regardless of syndecan-1 transgene expression. However, when grown in soft agar, cells expressing increased levels of cell surface syndecan-1 or expressing soluble syndecan-1 exhibit enhanced colony formation. The results of invasion assays through matrigel-coated transwells demonstrate that cells expressing either form of the syndecan-1 transgene show an improved invasive capacity as compared to controls. Thus, surprisingly, increased expression of syndecan-1 in breast tumors may contribute to enhanced growth, metastasis and a poor prognosis.

APPENDIX

Please see the attached manuscript entitled: "Increased syndecan-1 expression promotes anchorage-independent growth and invasion of MDA-MB-231 breast cancer cells."

Increased syndecan-1 expression promotes anchorage-independent growth and invasion of MDA-MB-231 breast cancer cells.

Carla Y. Pumphrey, Allison M. Theus, and Ralph D. Sanderson Arkansas Cancer Research Center, Departments of Pathology and Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas, 72205, USA

Running title: Syndecan-1 promotes growth and invasion

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Requests for reprints addressed to: Ralph D. Sanderson at Department of Pathology, Slot 517, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, Arkansas, 72205, USA, Telephone: (501) 686-6413; FAX: (501) 686-5168; email: sandersonralphd@uams.edu

The abbreviations used are:

Abstract

Syndecan-1 is a cell surface heparan sulfate proteoglycan that plays a role in regulation of complex cell behaviors including growth and invasion. How syndecan-1 affects cell behaviors is determined by multiple factors including level of syndecan-1 expression. To determine if increased expression of syndecan-1 on the cell surface or the production of soluble syndecan-1 affects breast tumor cell behavior, the MDA-MB-231 cell line was transfected with vector or either of two syndecan-1 gene cassettes and cloned cell lines were established. One cassette encodes the full-length cell surface form of syndecan-1 and other encodes a truncated molecule composed of the ectodomain which is secreted from cells in a soluble form. MTT analysis shows little difference in cell viability at several time points regardless of syndecan-1 transgene expression. However, when grown in soft agar, cells expressing increased levels of cell surface syndecan-1 or expressing soluble syndecan-1 exhibit enhanced colony formation. The results of invasion assays through matrigel-coated transwells demonstrate that cells expressing either form of the syndecan-1 transgenes shows an improved invasive capacity as compared to controls. Thus, increased expression of syndecan-1 in breast tumors may contribute to enhanced growth, metastasis and a poor prognosis.

Introduction

The cell surface heparan sulfate proteoglycan syndecan-1 regulates normal and cancer cell behaviors by binding growth factors, and by mediating cell adhesion and invasion [1]. Many tumors display altered syndecan-1 production and it has been suggested that the loss of syndecan-1 expression contributes to a malignant phenotype[2,3]. However, in multiple myeloma and pancreatic adenocarcinoma, syndecan-1 cell surface expression is high despite a large tumor burden and dissemination of the tumor cells [4,5]. Therefore, the role of syndecan-1 in the progression of a specific cancer is not inherently predicable and likely involves many factors including the type of cancer, the fine structure of syndecan-1, the level of expression on the cell surface and the amount of syndecan-1 shed from the cell surface.

Depending on the specific model used, syndecan-1 can reduce or promote tumor cell growth. Increased expression of cell surface syndecan-1 in a murine lung squamous cell carcinoma cell line results in improved proliferation [6]. Multiple myeloma and plasma cell leukemia cell growth is reduced by the addition of purified soluble syndecan-1 *in vitro* [7]. However, expression of a soluble syndecan-1 in the same plasma cell leukemia cell line promotes tumor formation (Yang, 2002). In normal murine mammary epithelia, loss of cell surface syndecan-1 expression results in a nonepithelial morphology, anchorage-independent growth and invasion in collagen gels[8]. When transfected with the syndecan-1 gene, transformed murine mammary epithelial cells regain epithelial morphology and lose neoplastic growth[9]. Collectively, these studies suggest syndecan-1,

either on the cell surface or in a soluble form has a role in maintaining mammary epithelial morphology and regulating growth and invasion.

Syndecan-1 also has a dual role in the metastatic potential of cancer cells. Cell surface syndecan-1 inhibits invasion of a plasma cell leukemia cell line through collagen gels[10]. However, expression of soluble syndecan-1 in these cells promotes cell invasion through collagen gels and these cells disseminate more readily *in vivo* (Yang, 2002). In breast cancer, immunohistochemical comparison of malignant and non-malignant breast tissue indicates a reduction in syndecan-1 expression on malignant cells within infiltrating ductal carcinomas[11]. In addition, syndecan-1 is found in the stromal compartment of infiltrating-ductal carcinoma but not in the stroma of normal breast tissue. Therefore changes in syndecan-1 expression in the stroma and by the malignant cells may promote breast cancer cell metastasis.

Many human breast cancer cell lines express abundant syndecan-1 on the cell surface (unpublished observation). This is unexpected because syndecan-1 expressed on the surface of murine mammary epithelial cell lines prohibits anchorage-independent growth and invasion. The MDA-MB-231 breast cancer cell line produces colonies in soft agar and is highly invasive, yet this cell line expresses cell surface syndecan-1. To evaluate the role of syndecan-1 in the basic characteristic tumor cell behaviors of anchorage-independent growth and invasion, we compared the syndecan-1 positive breast cancer cell line MDA-MB-231, with MDA-MB-231 cells engineered to express increased cell surface syndecan-1 or soluble syndecan-1. We found that these cells grow at a similar

rate in culture however, increased expression of cell surface syndecan-1 or soluble syndecan-1 improves colony formation in soft agar and invasion through matrigel-coated transwells. Therefore, increased syndecan-1 expression either on the cell surface of breast cancer cells or in a soluble form may contribute to a neoplastic phenotype.

Materials and methods

Cells and cell culture.

MDA-MB-231 cells were obtained from the American Type Culture Collection (ATCC; Rockville, MD). The cells were cultured in α-MEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin sulfate. ARK and ARP-1 cell lines were established at the Arkansas Cancer Research Center from bone marrow aspirates of multiple myeloma patients[4,12]. These cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin sulfate.

Construction of syndecan-1 expression vectors and establishment of transfected cell lines.

The human syndecan-1 cDNA cloned in a plasmid vector was a kind gift of Markku Jalkanen[13]. An oligonucleotide containing the coding sequence for a c-myc tag flanked by syndecan-1 gene sequences was produced for the purpose of inserting the c-myc tag into the syndecan-1 core protein. The oligonucleotide was designed such that the c-myc tag would be incorporated into the syndecan-1 ectodomain between amino acids 21 and 22 of the molecule. The c-myc tag oligonucleotide was incorporated into the syndecan-1

gene using a site-directed mutagenesis kit per the manufacturer's instructions (Clontech, Palo Alto, CA). Incorporation of the c-myc tag sequence was confirmed by DNA sequence analysis. The c-myc tagged syndecan-1 gene was used as a template for the PCR amplification of the full-length syndecan-1 open reading frame or a truncated syndecan-1 construct that lacks the syndecan-1 transmembrane and cytoplasmic domains (soluble syndecan-1). Forward and reverse primers incorporated BamHI and HindIII restriction endonuclease sites, respectively to allow for insertion of the PCR products into the pcDNA3.1- plasmid vector (Invitrogen, San Diego, CA) in the correct orientation. The reverse primer used to generate the soluble syndecan-1 gene cassette contains an in frame stop codon that ends translation after amino acid number 237. The integrity of the syndecan-1 and the soluble syndecan-1 gene cassettes was confirmed by DNA sequence analysis.

Subconfluent flasks of MDA-MB-231 cells were transfected with empty vector or vector containing the c-myc tagged syndecan-1 gene or the c-myc tagged soluble syndecan-1 gene using Lipofectin reagent according to the manufacturer's instructions (Invitrogen). Transfected cells were G418 selected and clones were isolated by limiting dilution.

Flow cytometry and immuno- and Western-blotting.

To confirm cell surface syndecan-1 expression, soluble syndecan-1 expression and to evaluate the size of syndecan-1 produced by these cells, MDA-MB-231 parental cells or transfected cell lines or conditioned media from these cells were evaluated. The cells were stained with FITC-labeled antibodies including an antibody specific for the human

syndecan-1 core protein (BB4), a c-myc specific antibody or an irrelevant control antibody. The results were determined by flow cytometry on a Becton-Dickinson (Mountain View, CA) FACScan using Cellquest 1.2 software. Conditioned media from parental MDA-MB-231 cells or the cloned cell lines was collected and evaluated by immuno-blot analysis and Western blot analysis using the BB4 anti-human syndecan-1 antibody as described previously[14,15].

MTT cell viability assay.

MDA-MB-231 parental cells, vector only trasfected cells and syndecan-1 or soluble syndecan-1 expressing clones were plated on 96-well plates in triplicate. Cell viability was determined following 0, 24, 48, and 72 hours in culture at 37°C in 5% CO_2 based on mitochondria conversion of MTT (Sigma, St Louis, MO) to formanzan as described previously (Pumphrey, 2002).

Anchorage-independent growth.

The wells of 24-well plates were covered with an agar solution composed of .5% SeaPlaque low melt agarose (FMC Bioproducts, Rockland, ME) in α-MEM, 20% fetal bovine serum. Gels were allowed to polymerize and then overlayed with a solution of .5% SeaPlaque low melt point agarose in α-MEM, 10% fetal bovine serum containing 5 x 10^3 cells in suspension. The gels were polymerized at room temperature, transferred to 37°C, 5% CO_2 and incubated for 14 days. The results were determined by counting colonies formed in duplicate wells and in triplicate experiments.

Invasion assay.

MDA-MB-231 parental cells or transfected cells were starved overnight in serum-free media containing .02% BSA and were seeded on reconstituted matrigel-coated transwells (8-μM pore size, Becton-Dickinson) at a concentration of 10^4 cells per well in the same serum-free media. The lower chamber contained NIH3T3 conditioned media as a chemoattractant. Following a 24-hour incubation at 37°C, 5% CO_2 the number of invasive cells was determined according to the manufacturer's instructions.

Results

Increase expression of syndecan-1 does not promote MDA-MB-231 cell viability in vitro.

MDA-MB-231 breast cancer cells were transfected with the plasmid vector pcDNA 3.1- or the same vector containing a syndecan-1 gene cassette encoding the full-length human syndecan-1 or a truncated syndecan-1 gene (Figure 1). The truncated syndecan-1 gene cassette lacks the DNA encoding the transmembrane and cytoplasmic domains therefore, expression produces soluble syndecan-1 that is secreted instead of incorporated into the cell membrane. MDA-MB-231 cells constitutively express syndecan-1 therefore, to distinguish native syndecan-1 from syndecan-1 produced by the transgenes, the gene constructs contain DNA sequence encoding a c-myc tag. Following G418 selection, clones were isolated by limiting dilution. To confirm the increased production of syndecan-1 on the cell surface, clones were stained with the BB4 antibody which is specific for the human syndecan-1 core protein or an antibody specific for the c-myc tag. The results were determined by FACS analysis and demonstrate a 6-9-fold increase in syndecan-1 core protein on the surface of the cells transfected with the full-length

syndecan-1 construct (Figure 2A. and data not shown). Immunoblot assay of the conditioned media of the clones indicates the expected increase in syndecan-1 found in the media of the cells transfected with the soluble syndecan-1 gene cassette (Figure 2B). Interestingly, shed syndecan-1 from the MDA-MB-231 vector only transfected cells was not detectible however, increased expression of syndecan-1 on the cell surface results in shedding. Western blot analysis reveals the presence of syndecan-1 in media conditioned by MDA-MB-231 clones expressing the syndecan-1 transgenes. Syndecan-1 from these cells appears as a high molecular weight smear similar to syndecan-1 found in other cell lines.

The level of syndecan-1 expressed on cells affects the interaction of syndecan-1 with growth factors. Therefore, cell viability assays were performed to determine if increased expression of syndecan-1, either on the surface of cells or in the media of the cells, affects MDA-MB-231 cell growth *in vitro*. Clones expressing increased amounts of syndecan-1 on the cell surface or expressing soluble syndecan-1 and vector only transfected parental cells were plated on 96-well plates and cell viability was determined at several time points by MTT assay. The results demonstrate no difference in cell growth regardless of syndecan-1 expression profile (Figure 3.).

Increased expression of syndecan-1 promotes anchorage-independent growth of MDA-MB-231 cells.

An *in vitro* hallmark of tumorigenic cells is anchorage-independent growth in soft agar. Therefore, we evaluated the affect of increased expression of syndecan-1 on the colony

formation of MDA-MB-231 cells in soft agar. The results indicate an increased number of colonies produced by the MDA-MB-231 cells that express increased cell surface syndecan-1 and cells that produce soluble syndecan-1 compared to vector only transfected cells (Figure 4.). When heparin is added to the soft agar, there is a decrease in colony formation regardless of level of syndecan-1 expression. The heparin likely sequesters growth factors thereby eliminating their availability to the cells resulting in decreased colony formation. Because heparin is a functional homologue of the heparan sulfate found on syndecan-1 this result suggests that the increased level of syndecan-1 and therefore heparan sulfate in close proximity to the cells may increase growth factor concentration near the cells thereby improving growth.

MDA-MB-231 cells expressing increased levels of syndecan-1 are more highly invasive. Cell surface syndecan-1 expression has been shown to block the invasion of the plasma cell leukemia cell line ARH-77 however, expression of soluble syndecan-1 in these same cells promotes invasion. MDA-MB-231 breast cancer cells are highly invasive, therefore, the affect of increased expression of syndecan-1 by these cells was evaluated. The results demonstrate improved invasive capacity of the MDA-MB-231 cells expressing either increased levels of cell surface syndecan-1 or cells that produce soluble syndecan-1 (Figure 5.).

Discussion

Cell surface molecules and components found in the extracellular matrix influence cell behaviors. The heparan sulfate proteoglycan syndecan-1 is found on many cell surfaces

and is the predominant cell surface proteoglycan on mammary epithelia[16,17]. Cell surface syndecan-1 and soluble syndecan-1 modulate interactions with any of a number of ligands and it is these interactions that influence complex cellular processes such as growth and migration[18]. In this study, we have evaluated the affects of increased syndecan-1 expression on the cell surface or in a soluble form on the characteristic tumor cell behaviors of the breast cancer cell line MDA-MB-231. In contrast to results of previous studies of syndecan-1 expression on murine mammary epithelia, we found that increased syndecan-1 expression promotes a malignant phenotype.

Studies of murine mammary epithelia and murine mammary tumor cells suggest that syndecan-1 expression on the cell surface prevents anchorage-independent growth and invasion[8,9]. However, human breast cancer cell lines, such as the MDA-MB-231 cell line, produce abundant syndecan-1 on the cell surface (Figure 2A) but also produce colonies in soft-agar and are highly invasive. Moreover, a 6-9 fold increased level of syndecan-1 expression on the cell surface promotes anchorage-independent growth and invasion through matigel (Figures 4 and 5). These results are inconsistent with the notion that cell surface syndecan-1 generally acts as tumor suppressor on cancer cells but highlights the differing roles that syndecan-1 may play depending on the type of cancer cell and the environment.

There are at least two possible reasons for the improved malignant phenotype displayed by the MDA-MB-231 cells expressing increased cell surface syndecan-1 or soluble syndecan-1. First, increased syndecan-1 core protein production may cause changes in

the fine structure of the heparan sulfate chains attached to the core protein and thus change the activities of the molecule. When the MDA-MB-231 parental cells and cells expressing increased levels of syndecan-1 on the cell surface were stained with an antibody specific for heparan sulfate chains, there was no difference in staining (data not shown). This result indicates that even though there is a 6-9 fold increase in cell surface syndecan-1 core protein there is not an increase in the cell surface level of heparan sulfate. This suggests that the syndecan-1 core protein on the transfected cell lines has either fewer or shorter heparan sulfate chains attached. Second, increased extracellular syndecan-1 produced as a result of transgene expression may affect cell behavior. The parental MDA-MB-231 cell lines produce little if any soluble syndecan-1 (Figure 2B). However, expression of increased levels of syndecan-1, including syndecan-1 incorporated on the cell surface, results in a great increase in syndecan-1 in the extracellular compartment. Indeed, differences in MDA-MB-231 cell surface heparan sulfate fine structure or the addition of extracellular heparan sulfate influences the mitogenic and migratory activities of fibroblast growth factor-2 on these cells[19,20].

This study indicates that high-level expression of syndecan-1 on breast cancer cells or the presence of abundant extracellular syndecan-1 may promote tumor progression. This is consistent with the finding that syndecan-1 is required for *Wnt-1* induced murine mammary tumor formation and the observation that syndecan-1 is present in the stroma of human infiltrating ductal carcinomas but not normal stroma[21,11]. The mechanism of syndecan-1 activity is unknown however, the role that syndecan-1 plays seems to be active because variation in syndecan-1 level or presence in the extracellular milieu affects

breast cancer cell behaviors. Therefore, therapies designed to block syndecan-1 activities or shedding could block breast cancer cell growth and metastasis.

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Figure Legends

Figure1. Structure of the c-myc tagged human syndecan-1 proteoglycans. Expression of the full-length syndecan-1 construct results in the production of cell surface syndecan-1. A soluble form of syndecan-1 devoid of the transmembrane and cytoplasmic domains is produced by expression of the truncated syndecan-1 construct. Syndecan-1 is composed of a core protein (open rectangle) containing a signal sequence (gray box), five potential glycosaminoglycan binding sites (lines) a transmembrane domain (lined box) and a cytoplasmic domain (black box). A c-myc tag (hatched box) was incorporated 3' to the signal sequence near the N-terminus of the core protein. Numbering indicates amino acid position.

Figure 2. Transgene expression increases levels of cell surface syndecan-1 and soluble syndecan-1. (A) Parental or transfected MDA-MB-231 cells were stained with an irrelevant type-matched control antibody (negative control, not shown) or antibody specific for the syndecan-1 core protein. The presence of syndecan-1 on the cell surface was determined by FACS analysis. MDA-MB-231 parental cells and MDA-MB-231 cells transfected with vector only are labeled 231 and 231neo, respectively. Clones of cells transfected with the full-length syndecan-1 construct are denoted as syn-1.B4, C4 and D4 and clones expressing the soluble syndecan-1 construct are labeled ssyn-1.C7, D5 and E4. The shift in fluorescence indicates the amount of cell surface syndecan-1. (B) Syndecan-1 was detected in the conditioned media of transfected MDA-MB-231 cells but not in conditioned media from vector only transfected cells. Conditioned media were analyzed by immune-dotblot using the human syndecan-1 specific BB4 antibody. ARP-1

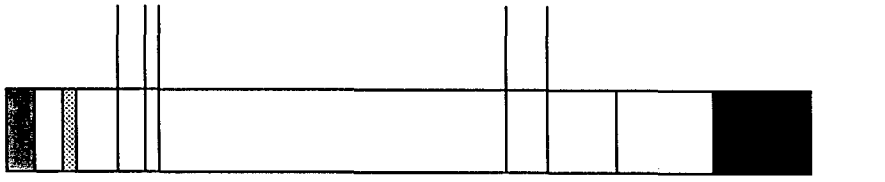
cell conditioned media serves as a positive control. (C) Western blot analysis of conditioned media demonstrates the production of high molecular weight soluble syndecan-1 by cells expressing either syndecan-1 transgene. Heparitinase digestion revels the syndecan-1 core protein devoid of heparan sulfate chains.

Figure 3. Increased expression of syndecan-1 does not affect cell viability in culture. Transfected MDA-MB-231 cells were plated in 96-well plates, incubated at 37°C, 5% CO_2 and at 0, 24, 48, and 72 hours cell viability was determined by MTT assay. Cell lines evaluated include: MDA-MB-231 vector only (circle), syn-1.B4 (square), syn-1.C4 (triangle), syn-1.D4 (upside-down triangle), ssyn-1.C7 (diamond), ssyn-1.D5 (hexagon) and ssyn-1.E4 (circle with dot). Results show means of triplicate wells and duplicate experiments +/- standard deviation.

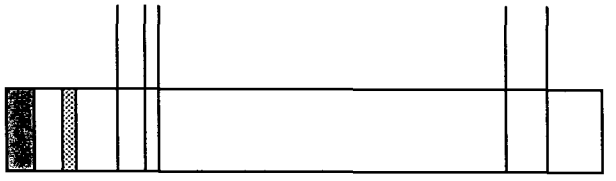
Figure 4. Increased expression of syndecan-1 promotes anchorage-independent growth. Colony formation in soft agar was evaluated for vector only transfected and syndecan-1 construct expressing MDA-MB-231 cells. Assays were performed with and without the addition of 10 μg/ml heparin. The results are shown as means +/- standard deviation of duplicate wells and triplicate experiments.

Figure 5. Increased syndecan-1 expression promotes an invasive phenotype. Invasion through matrigel-coated transwells was determined for vector only or syndecan-1 construct transfected MDA-MB-231 cells. The number of invasive cells is reported as a mean +/- standard deviation of duplicate wells and triplicate experiments.

Figure 1.



Full-length



Truncated

Signal sequence

C-myc tag

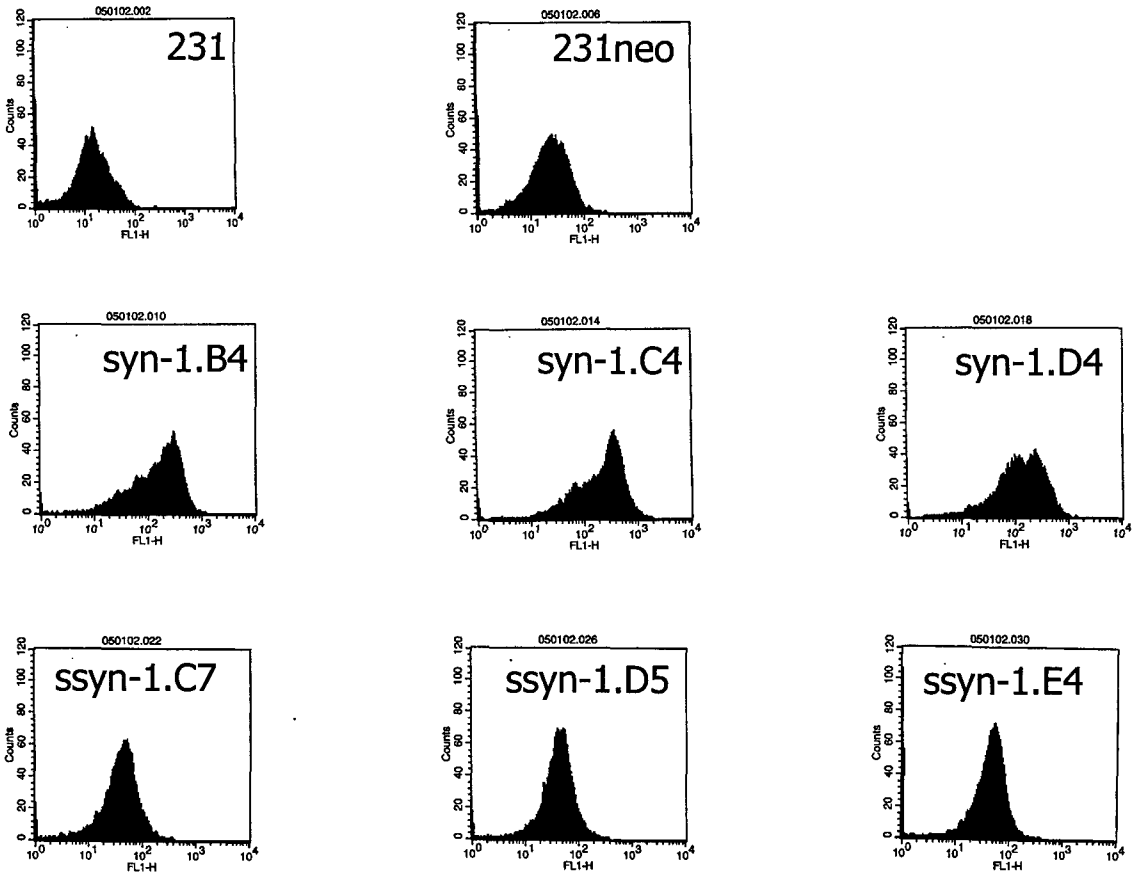
Transmembrane domain

Core protein

GAG

Cytoplasmic domain

Figure 2A.


050102.002
231
050102.006
231neo
050102.010
syn-1.B4
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syn-1.C4
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syn-1.D4
050102.022
ssyn-1.C7
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ssyn-1.E4
Counts
FL1-H

Figure 2 B and C

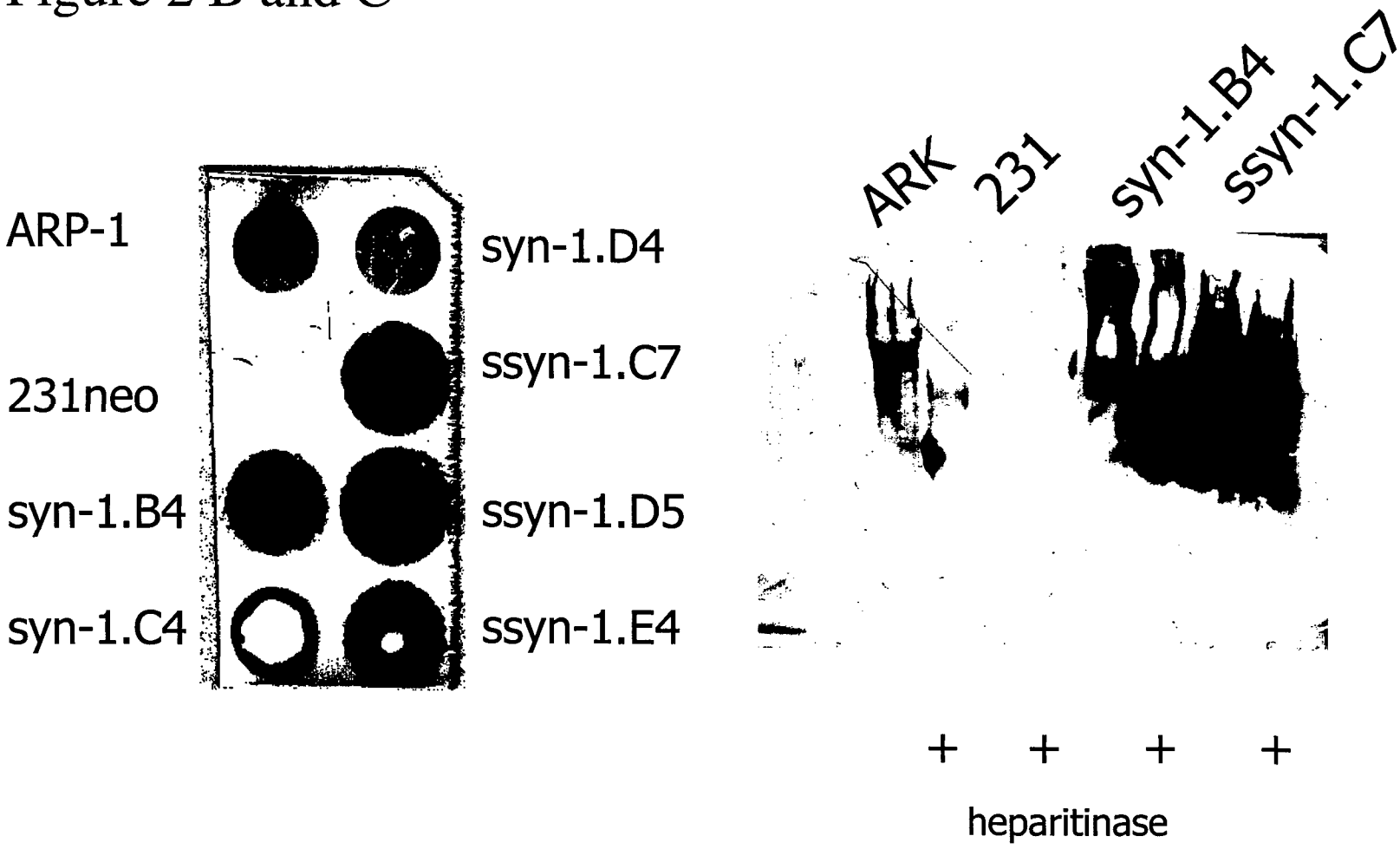


Figure 3.

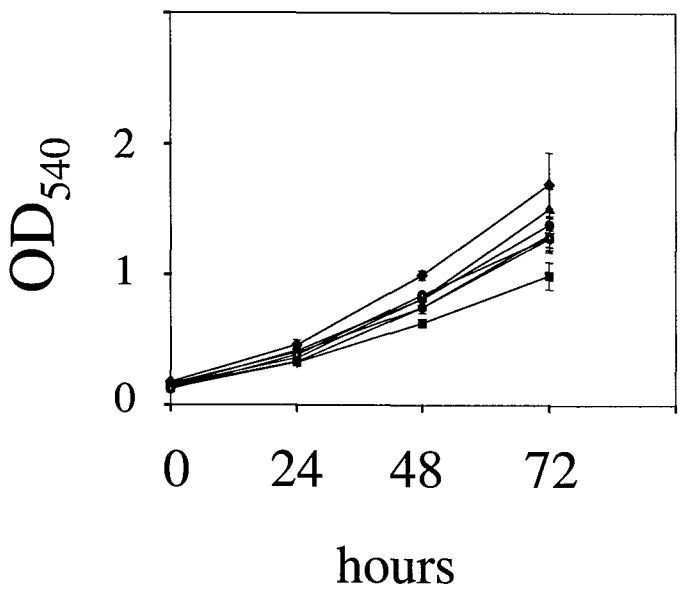


Figure 4

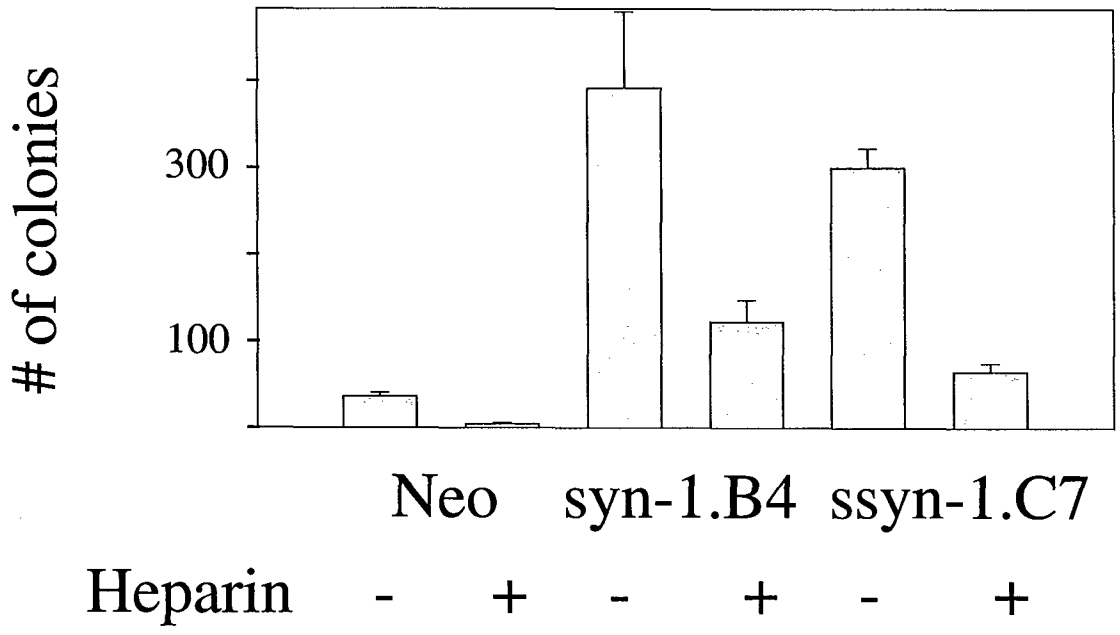
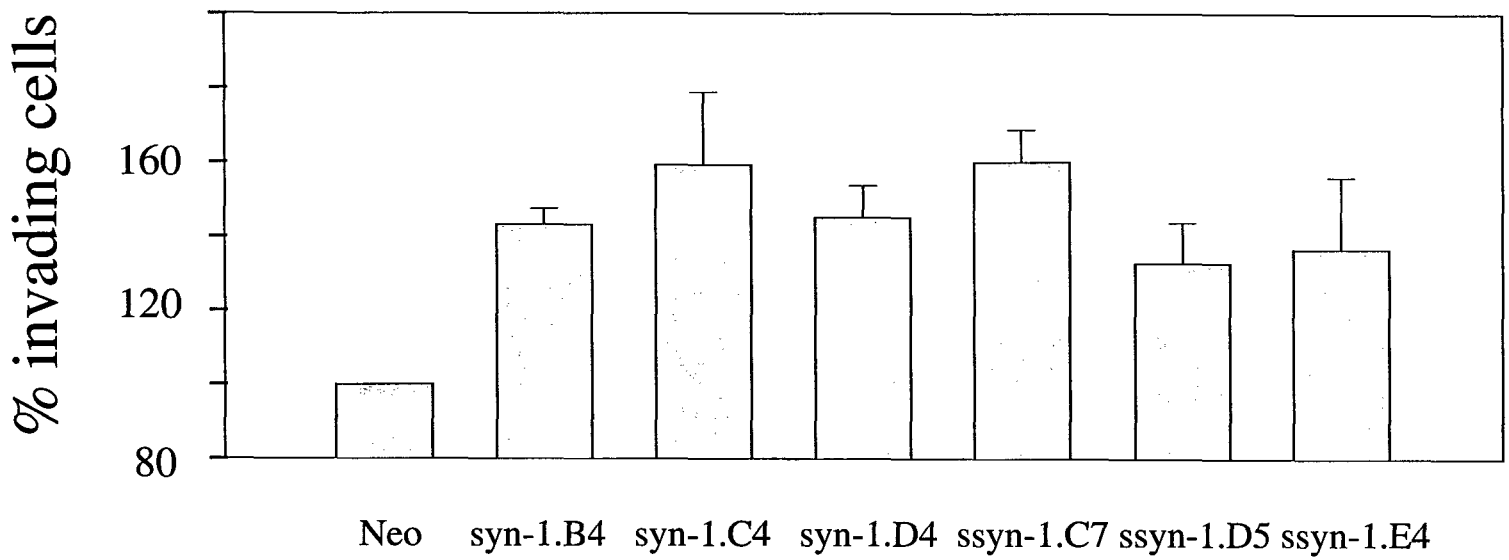


Figure 5


% invading cells
160
120
80
Neo
syn-1.B4
syn-1.C4
syn-1.D4
ssyn-1.C7
ssyn-1.D5
ssyn-1.E4

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504 SCOTT STREET
FORT DETRICK, MD 21702-5012

REPLY TO
ATTENTION OF

MCMR-RMI-S (70-1y) 10 Jun 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
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